IDENTIFICATION OF NORWEGIAN SPRING SPAWNING HERRING (*CLUPEA HARENGUS* L.) LARVAE FROM SPAWNING GROUNDS OFF WESTERN NORWAY APPLYING OTOLITH MICROSTRUCTURE ANALYSIS

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After the stock collapse in the 1960s the main spawning grounds of Norwegian spring spawning herring have been located off the Møre coast (northern grounds) and after 1989 spawning has also occurred at previous important grounds off the south-western coast of Norway (southern grounds). An overall objective of this study is to identify offspring from the different main spawning grounds in their nursery areas several months later. This requires criteria for identification of larval components from the main hatching areas. Peak hatching time at the two main spawning areas differ by approximately one month, being earlier at the northern spawning grounds. Passive drift from the southernmost areas adds another 2-3 months to this time difference until the larvae from the southern spawning grounds reach the spawning grounds off Møre. Otolith microstructure analysis of larvae collected along the drift path of the separate larval populations are used to trace past growth patterns. The results show a significant difference in back calculated increment width at age and in back calculated hatching distribution, and indicate that otolith microstructure can be used to separate components of offspring from the two spawning grounds.

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INTRODUCTION

The stock of Norwegian spring spawning herring collapsed in the late 1960s (DRAGESUND & al. 1980). Changes in the migratory pattern of the stock followed, but already some years before the actual collapse, the spawning had gradually moved towards northern grounds. The historical important spawning grounds off south-western Norway were abandoned in 1959, and subsequently the spawning has mainly occurred at northern grounds off Møre and less frequently off northern Norway (DRAGESUND & al. 1980; RØTTINGEN 1990; BERGSTAD & al. 1991). In 1989 when the strong 1983 year class recruited to the spawning stock, spawning was observed at the grounds off south-western Norway for the first time since 1959 (RØTTINGEN 1990). At present, less than 5 % of a total stock of 2.8 mill tons (ANON 1994) is spawning at the southern grounds (JOHANNESSEN & al. 1995).

The herring migrating southwards from the winter areas will first reach the northern spawning grounds and a few weeks later they will reach the southern spawning grounds. Despite the relatively small amount of eggs deposited at the southern grounds at present, it is interesting to study the significance of these grounds in the rebuilding process of the stock. Otolith microstructure analysis is a tool which potentially can be used in such studies. Since PANELLA (1971) discovered daily increments in fish otoliths, many studies have been conducted using otolith microstructure analysis to obtain knowledge about the early life history of fish (MCGURK 1984; CAMPANA & al. 1987; WATANABE & al. 1988; YOKLAVICH & BAILEY 1990; FOSSUM & MOKSNESS 1993) and its importance in fishery research is increasing. The fish otolith contains information about the environmental conditions which the larvae have experienced. For example one can back calculate larval growth and age (GEFFEN 1982; LOUGH & al. 1982; MOKSNESS & WESPESTAD 1989). By applying otolith microstructure analysis it is possible to separate larvae which have grown under different environmental conditions, and this has been done examining spring- and autumn spawned herring larvae (FOSSUM & MOKSNESS 1993). ZHANG & MOKSNESS (1993) have shown that juvenile otoliths can be treated to expose larval growth pattern and this extends the time period during which the fish can be separated.

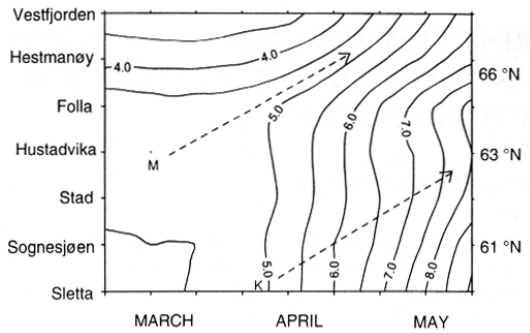

Fig. 1. Average surface temperatures in March-May along the Norwegian coast based on average values from 1936-1970 (data from MIDTTUN 1975). Dashed lines represent possible drift of larvae from the northern Møre component (M) and the southern Karmøy component (K).

FOSSUM & MOKSNESS (1995) measured significant differences in hatching distribution and otolith increment width at age for larvae of Norwegian spring spawning herring sampled from two patches, one north of 66° N and one south of 64° N. They suggest that the two patches are from northern and southern spawning grounds respectively. The difference in hatching distribution is expected since the mature herring reach the northern spawning grounds before they reach the southern. When studying the temperature regimes the two components of larvae experience while drifting from the spawning grounds (Fig. 1), we see that the southern component is drifting in an environment of increasing temperatures. This difference in temperature is expected to result in differences in somatic growth rate, and differences in otolith increment width at age. This can thus be used to identify the larval components.

In this study, otolith microstructure analysis is applied to characterise larvae from two of the main spawning grounds of Norwegian spring spawning herring. The aim is to consider if such information can be used to separate the components from these spawning grounds at a later stage.

MATERIAL AND METHODS

Herring larvae were mainly sampled at two transects off the west coast of Norway (Bømlo Transect, B1-B5 and Fedje Transect, F1-F5, Fig. 2) each with five stations (five nautical miles apart). In addition three stations were sampled west off Florø (E1-E3 Fig. 2) in an eddy observed by satellite data, and from information of the drift pattern of an Argos buoy (A. Johannessen, pers. commn). These stations were all located in the area between the northern and southern spawning grounds.

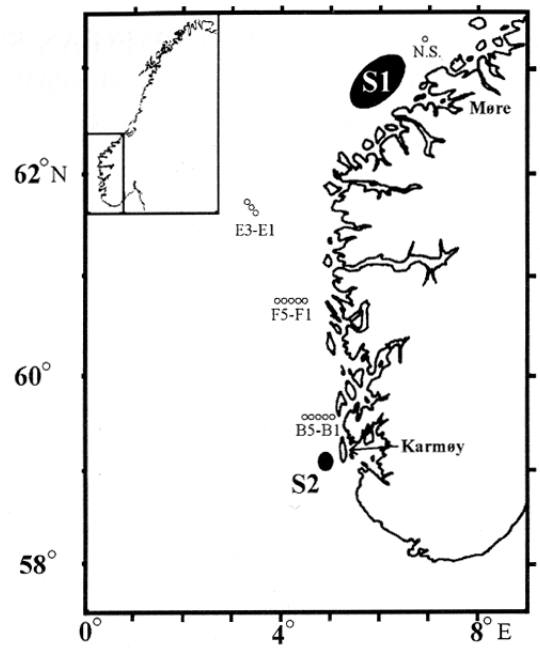

Fig. 2. Map of southern Norway with the northern (S1) and southern (S2) spawning grounds indicated. Sampling stations: E3-E1 = eddy samples; F5-F1 = Fedje samples; B5-B1 = Bømlo samples; N.S. = northern sampling stations.

Since herring larvae drift along with the northward flowing Norwegian coastal current, it is likely that the larvae caught in this area hatched at southern grounds. The sampling cruises were conducted in the period 5-23 May 1993.

Larvae were also sampled at stations north of the northern spawning grounds 20 May 1993. Data from an Argos drifting buoy released at the southern spawning ground west of the island Karmøy 15 April indicated that the southern larvae could not have drifted this far north by late May. It is therefore assumed that the larvae caught here, hatched at northern spawning grounds.

Four sampling nets were used. T-80 (ELLERTSEN & al. 1984) is a conical dip net with 0.5 m^2 opening and 375 µm mesh size. This net was mostly used to sample small larvae and was hauled vertically from 150 m (or 10 m above bottom when depth was less than 150 m) to the surface. The Gulf III (ZIJLSTRA 1970) is a high speed sampler which was towed behind the vessel cruising at five knots. It was lowered to a depth of 60 m and then hauled to the surface. The mesh size was 375 µm and it was used throughout the sampling period. The MIK sampler (MUNK 1988) is a trawl with a 2 m- (diameter) opening. It was used the same way as the Gulf III sampler but at a slower vessel speed (3 knots). Mesh size was 375 µm. This is an efficient sampling trawl for catching large larvae (MUNK 1988) and was used during the last cruise. Harstad trawl is a 10x10 m pelagic trawl with a mesh size of 500 µm in the cod end (GODØ & al. 1993), and it was operated the same way as the MIK.

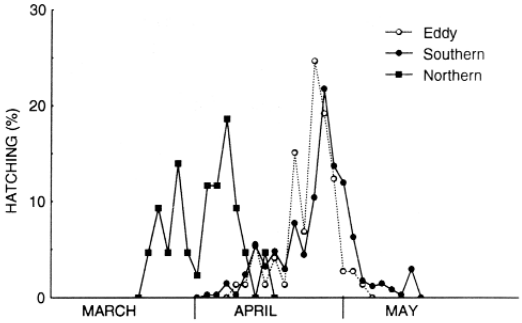

Fig. 3. Back calculated hatching curves for the three groups of larvae.

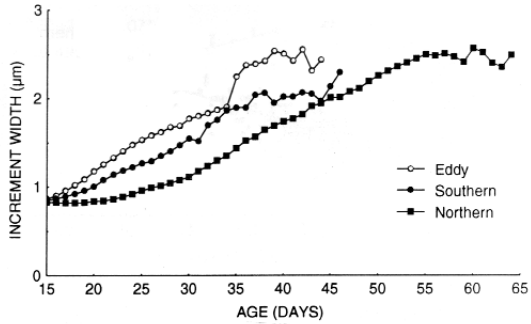

Fig. 4. Average increment widths in the otoliths at age for the three groups of larvae.

Four cruises were carried out sampling southern larvae, 5 May (Bømlo Transect, T-80 and Gulf III), 10 May (Fedje Transect, Gulf III), 18 May (Fedje Transect, Gulf III), 22-23 May (both transects in addition to stations in the eddy, Gulf III and MIK). The northern larvae were sampled 20 May using Harstad trawl. A maximum of 20 larvae from each haul were used for otolith examination and sub sampling was carried out with a plankton splitter when samples were larger. A total of 328 larvae were used for otolith examination in the study. During the first two cruises some of the larvae were too young to be aged by otolith analysis. To get correct back calculated hatching curves the age of 109 yolk sack larvae from the early cruises were in addition determined according to Sætre & al. (1987) using developmental stages (Doyle 1977).

The larvae were sorted out of the sample immediately after catch and preserved in 96 % ethanol (Brothers 1987; Butler 1992). The larvae standard length (SL) was measured and the otoliths were dissected out. The SL was not corrected for shrinkage. The sagittae were used for otolith examination (Secor & al. 1992). Both sagittae were mounted on glass slides using clear nail polish (Moksness & Wespestad 1989), and examination of the otoliths was carried out as described by Andersen & Moksness (1989), and Butler (1989) under 1000 X magnification. The examination of daily increment widths was carried out along the longest radius, and daily increments were counted and measured starting at the hatch check (Methot 1981; Campana 1992). The first increments after the hatch check were often difficult to see with the applied apparatus and the increments were then set to be 0.8 µm (because this was the width measured when visible).

The collected material was classified into the following groups, 1: the eddy sample, 2: the other southern sample, and 3: the northern sample. One criteria applied for separating larvae from different spawning grounds was the age of the larvae. The larvae were aged by counting the otolith increments and adding 10 days to compensate for the yolk sac period (Andersen & Moksness 1989). Kolmogorov-Smirnov (K-S) nonparametric tests were used to test for significant differences in hatching date frequencies. Increment width at age was another criteria used to separate the larvae components. The average increment width at age were calculated for the three groups and tested for differences using t-test corrected with Bonferroni's method (Johnson & Field 1993). This was done by dividing the level of significance (0.05) by the number of tests performed and thereby making the test more conservative. Growth curves for the groups were also back calculated by first establishing a relationship between SL (in mm) and otolith radii (OR, in µm) for each group.

RESULTS

The back calculated hatching curves (Fig. 3) show an overlap between the group from the eddy and the other southern group with mean hatching date being 23 and 25 April, respectively. The average hatching date for the northern group was 30 March. Comparing the northern group with the two southern groups gave significant differences (K-S-test, $D > 0.93$, $p < 0.001$).

There were differences in average increment width at age between the groups (Fig. 4). At age 15 days the curves were similar and the average increment width were just below 1 µm. From age 15 days the curves splitted into three components, the eddy group, the southern group and the northern group. The eddy group had the widest increments throughout the period examined for this group, and there was a clear increase at age 34 days to about 2.3 µm/day. The southern group had narrower increments and did not show the same jump at age 34 days. The narrowest increments were observed in the northern group. Differences in increment widths were found comparing the northern group with the eddy and southern group respectively (t-test, $p < 0.05/12$), Table 1), and also between the eddy and southern group (t-test, $p < 0.05/12$).

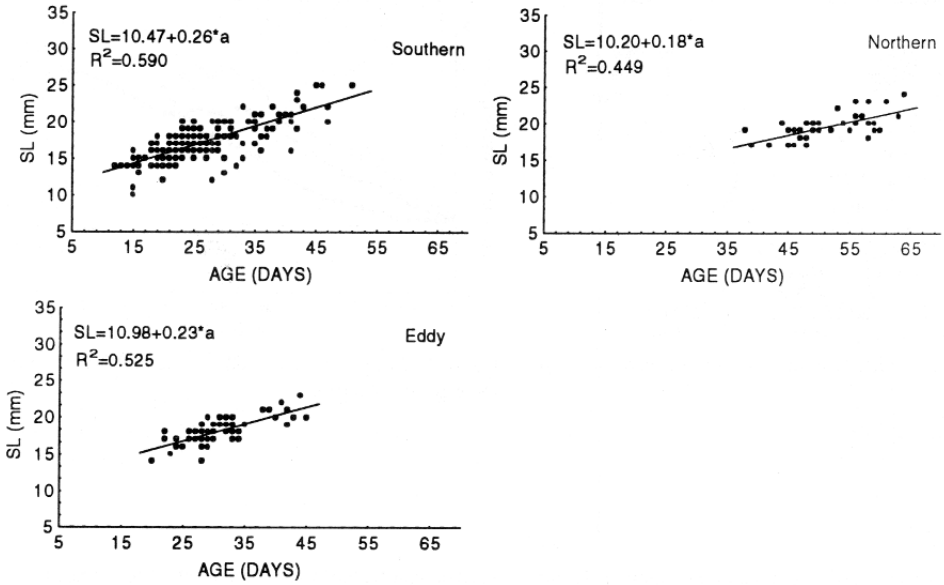

Fig. 5. Standard length versus age for all larvae of the three groups.

Standard length at age are presented as scatter plots for each group (Fig. 5). Tests for differences in slopes were performed and differences were found between the southern and northern group (t-test, $p < 0.05/3$).

Relationships between SL and OR were established, and a linear relation between ln(OR) and SL gave the best fit (highest coefficient of determination, R^2, Fig. 6).

There was a decline in back calculated growth rate from age 15 to 20 for all groups (Fig. 7) and the highest growth rate was found in the northern group (0.6 mm/day). From age 20-30 days the eddy group had the highest growth rate (about 0.5 mm/day). At the end of the age period the northern larvae had a growth rate of 0.3 mm/day.

Table 1. Results from t-tests comparing average increment widths at age 20, 25, 30, 35 days post hatching. Significance level, 0.05 and 0.01 were corrected for using Bonferroni's method resulting in new significance levels of 0.05/12 = 0.0042 and 0.01/12 = 0.00083, n.s.: not significant, **: $p < 0.01/24$.

	Age (days)			
Groups compared	20	25	30	35
Southern/Eddy	**	**	n.s.	**
Southern/Northern	**	**	**	**
Eddy/Northern	**	**	**	**

DISCUSSION

Earlier studies have applied otolith microstructure analysis to identify components of larvae (Fossum & Moksness 1993; Munk & al. 1991; Moksness 1992a), and they all show that such analysis may be a useful tool in recruitment studies. This study is the first to use this tool to identify different components of Norwegian spring spawning herring, a stock which is in the recovery phase after the collapse in the late 1960s.

For identification of spawning components, samples are required of all components which are identified a priori. We have to know which component we are analysing to establish the criteria for identification. This, we believe is achieved by sampling in areas were only one of the components could be present at the time of sampling. Four different nets were used in sampling, and this should be corrected for in quantitative analysis (Sommerton & Kobayashi 1989). Samples were taken both at night and daytime and this should also be corrected for in quantitative studies (Johannessen & Moksness 1991). However, this study had no aim of being quantitative and the principal aim of sampling was to get a broad sample of the composition of the larvae present in the investigated area at the time of sampling. We believe that this aim to a large extent has been achieved by selecting sampling nets according to the expected size of the larvae; T-80 early in the sampling period, when larvae

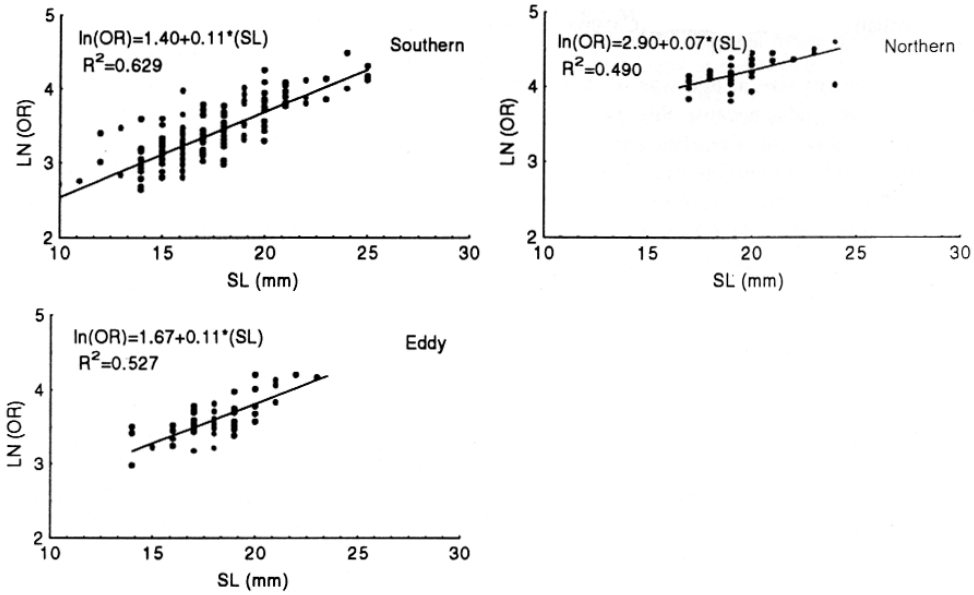

Fig. 6. Relationship between ln otolith radius (OR) and standard length (SL) for all larvae from the three groups. The equation of the relationship and the coefficient of determination, R^2, is given.

are expected to be small, MIK and Harstad trawl later in the sampling period when larvae are expected to be bigger, and Gulf III throughout the sampling period.

The relationship between number of otolith increments and age of herring larvae has been examined by Moksness (1992b), who concluded that the increment formation is daily. There have also been conducted studies to test the accuracy and precision of otolith analyses (Campana & Moksness 1991).

The average back calculated hatch dates for the northern group being 30 March is very similar to those obtained by Fossum & Moksness (1992) for the years 1985 and 1989, and by Fossum & Moksness (1993) for the year 1990. The 1991 year class though had a different average hatching date, 18 March (Fossum & Moksness 1995). This suggest relatively stable year to year hatching periods. Back calculated hatching curves in this study suggest that there is a one month time lag between the hatching at southern grounds compared with northern grounds. This difference is most likely due to different migration distances from the wintering area to the two spawning grounds (Johannessen & al. in press). Therefore, one can in the future expect differences in the hatching distribution from the two grounds even if the overall hatching varies from year to year. Changes in migratory pattern may, however, alter this.

Campana & Jones (1992) suggested that back calculated hatching curves should be corrected for cumulative mortality. The reason for this is that the older larvae will have experienced a longer period with mortality than the younger larvae and may therefore be underestimated in the samples. Such corrections are not made here because this requires actual mortality data. Also, the samples, except those from the earliest Bømlo and Fedje Transects, are taken at least one month after hatching and therefore the significance of a correction would be relatively small since the mortality is expected to be smaller in this period

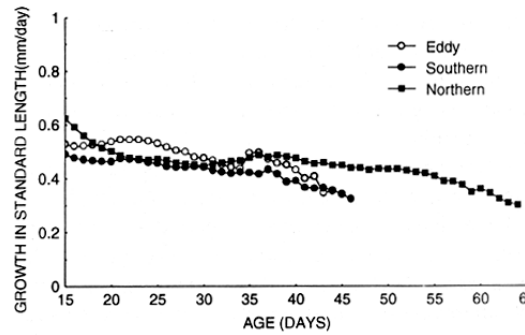

Fig. 7. Average back calculated somatic growth curves (standard length) for the three groups. The curves are based upon a relationship between otolith radius and standard length.

than shortly after hatching (Campana & Jones 1992).

Average otolith increment size at age was of most importance to the present study, because this was expected to be the best criteria for identifying the two components. The increment width is determined by factors such as temperature and prey situation (Moksness & al. 1995) and variability in these factors may result in different increment width pattern. The two components showed significant differences in average increment width at ages 20, 25, and 30 days. The eddy group, except from being different from the northern group, also had significantly higher increment widths than the southern group. These two groups were assumed to belong to the same component and the differences in increment widths suggest that the eddy larvae had experienced other conditions than the southern group. Studies from other areas have shown different larval feeding conditions inside eddies, than in the surrounding water masses (Canino & al. 1991) and this may explain the higher increment widths recorded in the otoliths of the eddy larvae. The average temperature history (Fig. 1) may explain the difference in increment width between the northern and the southern component. Controlled laboratory experiments are, however, needed to resolve the influence of temperature and food availability on otolith increment widths. There were large day to day variations in average increment widths for all groups when approaching the end of the available age period. This may be due to the decreasing number of larvae in the material through the age period.

Differences in environmental conditions may result in different accuracy of the age estimates. For example will the larvae have a longer yolk sac period at lower temperatures. The number of unreadable increments in the area after the hatch check will also be influenced by the environmental conditions. Therefore, the average increment width at age-curves may be skewed on the x-axis, and just testing for differences in average increment width at age, may be inadequate. A better way to test for differences in increment pattern will probably be to look at the slopes of the curves, and this may be applied in future studies. On the other hand, since the northern component experience the lowest temperatures, the age of these larvae will tend to be underestimated. This will skew the curve of average increment width at age for these larvae to the right and strengthen the differences already revealed by the tests.

The differences in the relationship between standard length and age between the groups were difficult to interpret. The northern group showed the lowest slope (although not significantly different from the eddy group) which was expected considering the otolith increment width data. Back calculated growth curves for standard length did not give the same picture as otolith increment growth. The growth in standard length showed that the eddy larvae had higher growth than the other groups from age 20 to 30 days, and that in the rest of the period the northern group had the highest growth. The basis for the growth curves were the relationship between otolith radius and standard length and the different groups contain larvae of different size groups. This will probably affect the relationship since larvae of different ages do not necessarily have the same relation between otolith radius and standard length. Therefore, to back calculate somatic growth rate, one should have a broad sample of age groups to base the calculation upon. At least, the age groups should not vary much between the groups analysed. Also, there may not be a simple linear relationship between standard length and otolith radius (Mosegaard & al. 1988; Secor & Dean 1989; Campana 1990) and this would affect the results. At the earliest ages all the growth curves showed high growth rates (over 0.8 mm/day). This may to some extent be a result of the difficulties of interpreting the inner part of the otoliths. If the inner increments were in fact smaller than the 0.8 μm they were set to be (and this may very well be the reason way they were not seen), this will tend to overestimate the growth at age. Therefore Scanning Electron Microscope (SEM) analysis would be preferred to obtain accurate data for the first days after first feeding. This problem, however, declines as the larvae grow, and after age 20 days there is no longer a problem to count and measure the increments.

The conclusion from this study is that otolith microstructure analysis is a useful tool in identifying components of larvae which have experienced different environmental conditions. There seems to be two criteria which are best. Hatch date analysis can be used in the early stages, but as the larvae grow, the age of the larvae becomes difficult to determine and hatch date analysis will then be less useful. Increment width pattern is assumed to be a better criteria and can be applied as long as the microstructure of the otolith can be analysed. Zhang and Moksness (1993) have shown that adult otoliths can be treated to expose the larval increment width pattern. If the larval increment widths shall be used to identify the origin of adult herring in the future one should build a data base where information on increment width at age for larvae from different spawning grounds is recorded every year. This is necessary to monitor possible year to year differences. Otolith analysis therefore has to be an annual event, and this would provide unique possibilities for studies of later stock composition and migrating pattern.

ACKNOWLEDGEMENTS

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